Award Number: W81XWH-16-1-0239

TITLE:
Lung Cancer-Specific Circular RNAs as Biomarkers

PRINCIPAL INVESTIGATOR:
Yin-Yuan Mo

CONTRACTING ORGANIZATION:
University of Mississippi Medical Center
Jackson, MS 39216

REPORT DATE:
August 2017

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2017	Annual	1 Aug 2016 - 31 Jul 2017

4. TITLE AND SUBTITLE

Lung Cancer-Specific Circular RNAs as Biomarkers

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0239

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Yin-Yuan Mo

E-Mail: ymo@umc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Mississippi Medical Center
2500 State Street, G652
Jackson, MS 39216

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The major goal of this application is to determine whether lung cancer cells differentially express circular RNAs such that these circular RNAs may serve as novel biomarkers for lung cancer diagnosis and prognosis. There are two Tasks in this application. First, we will perform microarray profiling to determine whether lung cancer cells display different patterns of circular RNAs from those of normal cells. Second, we will determine whether differential expression of circular RNAs can also be detected in cell culture models. Third, we will determine whether circular RNAs can be detected in circulation system. The success of this study may lead to novel clinical applications such as identification of biomarkers or therapeutic targets for lung cancer.

15. SUBJECT TERMS
lncRNA, circRNA, biomarker

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	7	USAMRMC

Table of Contents

Introduction

Circular RNAs (circRNAs) belong to a special group of long non-coding RNAs (lncRNAs). Although in some cases, circRNAs can code for protein, most of them lack the coding capacity. circRNAs are formed from lncRNAs or protein coding genes often through back splicing. There are five types of circRNA derived from: 1) exonic, 2) intronic, 3) antisense, 4) sense overlapping and 5) intergenic. "Exonic" represents circRNA arising from the exons of the linear transcript; "Intronic" represents the circRNA arising from an intron of the linear transcript; "antisense" represents circRNA whose gene locus overlap with the linear RNA, but transcribed from the opposite strand; "sense overlapping" represents circRNA transcribed from same gene locus as the linear transcript, but not classified into "exonic" and "intronic"; lastly, "intergenic" represents circRNA located outside known gene locus.

Despite different types of circRNAs, they all can play a regulatory role in gene expression through microRNA-mediated repression. Thus, we hypothesize that lung cancer may exploit this mechanism for its own advantage and as such lung cancer may display a very different circRNA pattern from normal lung cells Therefore, the major goal of this application is to determine whether we can identify differentially expressed circRNAs in lung cancer.

Body

CircRNAs are aberrantly expressed in lung cancer. As newly discovered molecules, circRNAs are poorly characterized. Little is known whether they are dysregulated in lung cancer. Thus, our first step was to characterize these new molecules by profiling. Results

indicate that over one hundred of circRNAs are either upregulated or downregulated. We listed four of them as representative differentially expressed circRNAs in Table 1.

Table 1, Representative circRNAs that are differentially expressed (tumor cells vs normal)

circRNA	Fold change	Regulation	Transcript	MRE1	MRE2	MRE3	MRE4	MRE5
hsa_circRNA_400633	8.8	up	NM_005063	hsa-miR-5589-5p	hsa-miR-1253	hsa-miR-3127-3p	hsa-miR-541-3p	hsa-miR-3691-3p
hsa_circRNA_101100	8.6	up	NM_007007	hsa-miR-183-5p	hsa-miR-383-3p	hsa-miR-433-3p	hsa-miR-607	hsa-miR-640
hsa_circRNA_005054	0.02	down	NM_001103184	hsa-miR-612	hsa-miR-6515-3p	hsa-miR-4753-5p	hsa-miR-362-5p	hsa-miR-450a-2-3p
hsa_circRNA_001831	0.03	down	NM_015356	hsa-miR-3151-5p	hsa-miR-6791-5p	hsa-miR-939-5p	hsa-miR-637	hsa-miR-7974

For example, hsa_circRNA_400633 and hsa_circRNA_101100 were upregulated with over an 8-fold increase in tumor vs normal with p value <0.05. On the other hand, the expression level of hsa_circRNA_005054 and hsa_circRNA_001831 was 0.02 and 0.03, respectively, as compared to normal cells as 1. To better illustrate how the circular form is formed, we provide the sequence for hsa_circRNA_400633 and hsa_circRNA_101100, as shown in Figs. 1 and 2 as an example. The top part is the actual sequence and the bottom part is when a circle is formed. Two ends at the junction were highlighted by green and blue, respectively.

aatgatgtctatgaatgggctcgtgaccaccgtgcccaccacaagttttcagaa
acacatgctgatcctcataattcccgacgtggcttttcttctctcacgtgggt
tggctgcttgtgcgcaaacacccagctgtcaaagagaaggggagtacgctagac
ttgtctgacctagaagctgagaaactggtgatgttccagaggag

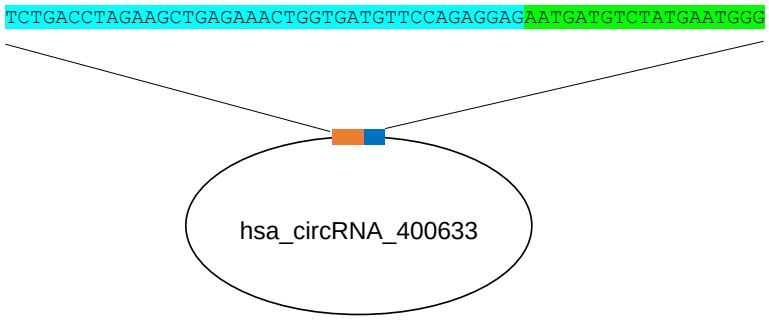

Fig. 1 DNA sequence of hsa_circRNA_400633. The junction of two ends is highlighted by red and blue, respectively.

CircRNAs can potentially target microRNAs. One of potential functions for circRNAs is the capability to serve as sponges to neutralize the endogenous microRNAs. In this regard, all of these circRNAs had the potential to target more than one microRNAs. We listed five microRNAs each for four representative circRNAs (Table 1). For example, miR-541-3p suppresses tumor progression by directly targeting TGIF2 in non-small cell lung cancer. On the other hand, miR-183 regulates autophagy and apoptosis in colorectal cancer through targeting of UVRAG; and it promotes proliferation and invasion in esophageal squamous cell carcinoma by targeting programmed cell death 4. It is evident that these microRNAs can serve as either oncogenes or tumor suppressors, thus impacting various aspect of tumorigenesis.

gaagggaaatggatactgcaagaacgccattgagtgaagctgaattttgaagaaatcatgaataga
aatagggcaatctcaagcagtgctatttcgagagctgtgtctgatgccagtgctggtgattatgg
gagtgctattgagacactggtaactgcaatttctttaattaaacaatccaaagtatctgctgatg
atcgttgcaaagttcttattagttctttgcaagattgccttcatggaattgagtccaagtcttat
ggttctggatcaagacgtgaacgatcaagagagagggaccatagtagatcacgagaaaagagtcg
acgtcataaatcccgtagtagagaccgtcatgacgattattacagagagagaagcagagaacgag
agaggcaccgggatcgtgaccgagaccgtgaccgagagcgtgaccgagagcgcgaatatcgtcat
cgtta

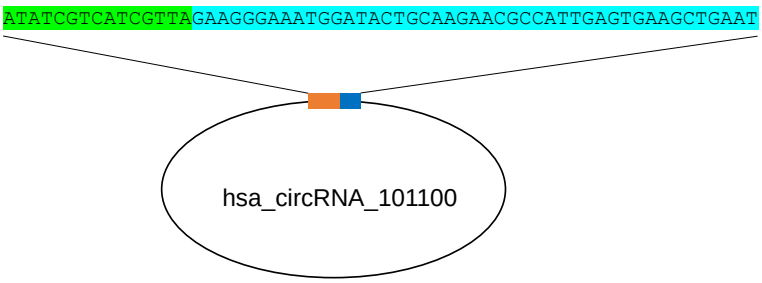

Fig. 2 DNA sequence of hsa_circRNA_101100. The junction of two ends is highlighted by blue and green, respectively.

Together, these findings suggest that aberrant expression of these circRNAs may affect the levels of these microRNAs, thus, contributing to lung tumorigenesis.

Key Research Accomplishments

- We identified over 100 upregulated or downregulated circRNAs from lung cancer cells through profiling.
- All of these identified circRNAs carry microRNA binding sites, through which they may regulate the level of endogenous microRNAs.
- In this regard, hsa_circRNA_400633 can potentially target miR-541-3p which has been shown to be a tumor suppressive microRNA.
- We will determine whether any of these circRNAs impact tumor cell growth in the cell culture models. We will also test whether they are differentially expressed in serum samples from normal and lung cancer patients such that they may serve as biomarkers for lung cancer.

Reportable Outcomes

Not yet.

Conclusions

Microarray profiling has identified over a hundred of upregulated or downregulated circRNAs from lung cancer cells. We are currently determining whether ectopic expression of any of these circRNAs will impact lung tumor cell growth and invasion. We will also determine these circRNAs can target the predicted microRNAs which in turns regulate their expression. Finally, we will test their potential as lung cancer biomarkers.